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ENDOCRINE AND METABOLIC RESPONSE TO SHOCK AND TRAUMA
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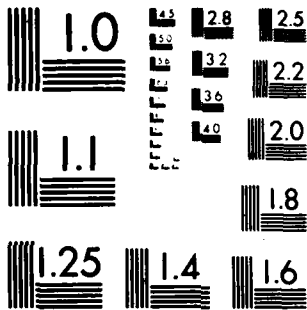
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ENDOCRINE AND METABOLIC RESPONSE TO SHOCK AND TRAUMA (U)

FINAL REPORT

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A second series of studies was performed to determine the existence of circadian rhythm of corticosteroids in baboons and rhesus monkeys. Adrenalectomized rhesus monkeys showed circadian and infradian cycles during a constant infusion of cortisol. These findings provide evidence for rhythmic degradation of cortisol which may contribute to the overall circadian dynamics of corticosteroids. A baboon model was developed, and stable control rhythms were obtained in several animals.

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In conducting the research described in this report, the investigator(s) adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council.

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I. Adrenal and Pancreatic Hormones and the Metabolic Response to Shock

The metabolic and endocrine response to major injury can induce a series of deleterious effects which play an important role in the prognosis of severely ill patients. Reversal of these aspects of the response by providing the required metabolic substrates and the means for their adequate utilization may promote a more rapid recovery. Management of the catabolic response to injury will best be obtained following identification of the controlling mechanisms and triggering signals involved in this sequence of events.

Hemorrhage evokes a marked secretion of the adrenal hormones which have metabolic effects similar to those observed after shock, and appeared to be likely contributors to the generation of the observed changes. Similarly, insulin and glucagon, with their profound effects on protein and carbohydrate metabolism, are considered contributors to the shock-induced imbalances of glucose and amino acid utilization.

In this series of experiments, a splenectomized-pancreatectomized-adrenalectomized monkey (SPAM) preparation was used to eliminate the effect of pancreatic and adrenal hormone responses to hemorrhagic shock. Three studies were carried out to document the effect of hemorrhagic shock on: 1) insulin resistance, amino acid metabolism and overall nitrogen balance in monkeys in varying nutritional states; 2) the role of glucagon in inducing protein catabolism in animals of varying nutritional status; 3) insulin resistance, muscle amino acid metabolism and nitrogen balance during constant cortisol and insulin infusions.

A. Effect of Nutritional Support

In order to abolish the influence of nutritional changes after shock, a group of 31 rhesus monkeys were divided into 4 groups; 2 groups to receive monkey chow ad libitum, the other to receive 80 cal/kg/day (normal for a monkey) as intravenously administered 50% dextrose plus freamine and electrolytes. Insulin was infused in an amount adequate for the maintenance of blood sugar levels below 200 mg per 100 ml. This regimen was begun a week prior to shock and continued during and after shock. One half of each nutritional group was subjected to removal of spleen, pancreas and bilateral adrenalectomy.

Although many of the biochemical alterations induced by hemorrhagic shock occurred in approximately the same degree in both intact and adrenalectomized-pancreatectomized monkeys, the probable effects of nutritional support obscured the significance of the metabolic responses in the two groups.

One parameter, shock-induced muscle insulin resistance, was not significantly altered by either the nutritional support or the absence of induced adrenal and pancreatic hormone levels. The response of isolated skeletal muscle to insulin is shown in Table I. Although the streptozotocin-treated and pancreatectomized groups are not strictly comparable, it can be seen that in both groups the tissue becomes unresponsive to insulin after shock. The pre-shock sensitivity to insulin appears to be depressed in the nutritional-supplemented animal, but since these experiments were done at separate times, this assertion cannot be validly made at this writing.

Leucine oxidation to CO_2 by isolated skeletal muscle (Table I) was the same in intact sham and endocrine-operated groups, although the increase was only of marginal significance in the nutritionally supported groups in which the post-shock increase in leucine oxidation appeared to be suppressed.

METABOLIC CONSEQUENCES OF HEMORRHAGIC
SHOCK IN THE RHESUS MONKEY

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TABLE I

Ab Libitum Fed

Intact	Adrenalectomized Streptozotocin diabetic	Intact	Adrenalectomized Pancreatectomized
(N=8)	(N=7)	(N=8)	(N=8)

Glucose Oxidation
(CPM/hr/mg)

PRESHOCK

Baseline	20 ± 13.6	21.4 ± 2.8	21.6 ± 3.1	14.5 ± 2.8
With Insulin ¹	90 ± 12.6	96.4 ± 3.5	36.5 ± 3.6	38.7 ± 3.5

4 HR POSTSHOCK

Baseline	24.3 ± 1.4	26.1 ± 2.4	21.1 ± 1.6	15.8 ± 2.8
With Insulin ¹	28.9 ± 2.6	20.7 ± 1.1	21.1 ± 1.6	21.4 ± 3.1

Leucine Oxidation to CO₂
(CPM/mg/hr)

<u>PRESHOCK</u>	12.0 ± 1.3	12.7 ± 2.3	8.4 ± 1.1	12.6 ± 3.2
<u>4 HR POSTSHOCK</u>	22.6 ± 3.2	26.0 ± 1.9	9.7 ± 0.9	13.4 ± 2.1

Leucine Incorporation
Into Protein (CPM/mg/hr)

<u>PRESHOCK</u>	24.1 ± 2.6	21.9 ± 3.1	13.5 ± 1.8	8.0 ± 2.1
<u>4 HR POSTSHOCK</u>	8.3 ± 1.6	7.9 ± 1.1	17.0 ± 2.7	23.3 ± 3.6

¹Insulin added to the incubation medium (24 MU/ML)

Leucine incorporation into protein responded to shock in a dimetrically opposed manner in the ad libitum fed versus the nutritionally supported group (see Table I). In the control and adrenalectomized animals consuming food ad libitum, shock reduced leucine incorporation into protein to about one-third control values, while in both groups of monkeys receiving intravenous nutrition, shock increased leucine incorporation into protein. Nutritional support appeared to slightly reduce the pre-shock leucine incorporation rate.

The animals receiving nutritional support were studied to determine nitrogen balance. Measurements corresponded with the reduced post-shock leucine oxidation and increased incorporation into protein, in that shock did not evoke a nitrogen catabolic response. The lack of a nitrogen losing phase after shock may have been partially the result of post-shock fluid retention, but certainly the large catabolic response usually observed after major trauma did not occur.

Determination of the significance of these findings will require further study, but it is possible that high glucose availability (or the high levels of insulin necessary to cover the glucose) may have prevented or modified the normal metabolic stimuli after shock. Further experiments will be necessary to better define the effects of shock on metabolism and the ability of nutritional influences to modify the usual response, as well as to determine the role of hormones in controlling these changes.

B. Role of Glucagon in Post-Operative Catabolism

Previous studies have related elevated glucagon levels to post-traumatic catabolism. Whether or not this catabolism results directly from inappropriate hypersecretion of glucagon remains controversial. Pozefsky's forearm study found no effect of glucagon on muscle protein catabolism; however, the systemic effect was not determined. Aoki and Cahill observed an unexpected anabolic

effect of infused glucagon and hypothesized a feedback inhibition of the alpha cell to explain it. This study was designed to eliminate variables of pancreatic and adrenal hormone secretion following surgery to assess the catabolic potency of glucagon.

Forty rhesus monkeys were randomly assigned to a low-calorie group receiving D5W (24 cal/kg/24 hr) and to a high-calorie group receiving D25-4% Freamine (80 cal/kg/24 hr). After 24 hours of infusion, total pancreatectomy, splenectomy and bilateral adrenalectomy were performed. Hydrocortisone (8 mg/kg/24 hr) and insulin (15 and 3 U/kg/24 hr respectively) were infused continuously. Glucagon was continuously infused at two different doses to 2 sub-groups of animals in each group to establish serum insulin/glucagon molar ratios of 4 and 0.4. A third subgroup received no glucagon. Splenectomized monkeys served as controls.

In well-alimented monkeys, low I/G molar ratios (0.4) were associated with high blood FFA and glucose levels; however, there was no significant increase in urinary nitrogen loss when compared to glucagon-deprived animals. Lack of exogenously administered glucagon in the pancreatectomized monkeys were associated with elevated blood alanine levels and decreased urinary excretion of nitrogen in the form of urea, alterations which were reversed by glucagon administration (Table II).

In the low-calorie group, low I/G ratios (0.4) were associated with significant increases in excretion of urinary nitrogen, when compared to glucagon-deprived animals; however, no significant effects on blood FFA or glucose levels were noted (Table II).

ROLE OF GLUCAGON IN POSTOPERATIVE CATABOLISM

TABLE II

	25% DEXTROSE - 4% FREAMINE (80 Cal/kg/24 hr)					5% DEXTROSE (24 Cal/kg/24 hr)				
	PREOP	POSTOP				PREOP	POSTOP			
		CONTROL	PANCREATECTOMIZED ADRENALECTOMIZED				CONTROL	PANCREATECTOMIZED ADRENALECTOMIZED		
I/G RATIO	-	-	No Glu- cagon	4.0	0.4	-	-	No Glu- cagon	4.0	0.4
GLUCOSE mg%	238 ±22	264 ±52	250 ±40	206 ±39	450 ±53	122 ±17	110 ±19	121 ±56	164 ±45	180 ±53
FFA uM/cc	.521 ±.06	.471 ±.08	.406 ±.07	1.40 ±.28	1.24 ±.15	1.19 ±.06	.982 ±.26	1.02 ±.23	.998 ±.36	.87 ±.04
ALANINE uM/cc	.59 ±.1	.35 ±.11	1.44 ±.10	.67 ±.05	.53 ±.11	.35 ±.11	.52 ±.03	1.30 ±.10	.80 ±.09	.54 ±.45
%UREA in URINE NITROGEN	88 ±4	82 ±3.5	63 ±3.1	85 ±5.7	85 ±2.7	82 ±4.2	88 ±3.4	65 ±4.1	81 ±4.2	91 ±4.1
NITROGEN BALANCE mg/kg/24 hr	±51 ±34	-97 ±36	-24 ±98	-115 ±40	-101 ±79	-419 ±34	-407 ±44	-240 ±18	-298 ±25	-381 ±59

[p 0.24]

[p 0.30]

[p .0124]

[p 0.05]

It was concluded that hyperglucagonemia does not directly cause significant nitrogen loss in post-operative primates receiving eucaloric nutritional support and that in post-operative primates receiving reduced calories, elimination of pancreatic glucagon is associated with decreased urinary losses in nitrogen.

Whether post-operative endogenous production of glucagon is appropriately high for the ongoing state of starvation remains to be determined.

C. Effect of Permissive Amounts of Insulin and Cortisol

Cortisol plays a major role in endocrine response to trauma and has been associated with protein catabolism. This study investigates the role of cortisol and insulin in generating changes in post-shock muscle protein metabolism and insulin resistance.

Ten rhesus monkeys were splenectomized, pancreatectomized (Ps) and adrenalectomized (Ax), placed in chairs and maintained with IV nutrient solution (80 cal/kg/day) and constant replacement of hydrocortisone and insulin. Ten control monkeys were splenectomized only and received similar IV alimentation. One week following surgery, half the animals in each group were reduced to 24 cal/kg/day (low-calorie group). On the eighth day, all monkeys were subjected to severe hemorrhagic shock (40 mmHg for 3 hours) with a resulting five-day mortality of 50%. The high-calorie group continued to receive 80 cal/kg/day throughout and following the shock period. Blood, urine and gastrocnemius muscle samples were collected before and 24 hours after shock to determine nitrogen balance (corrected for BUN), blood glucose, FFA and alanine levels and in vitro muscle leucine oxidation, protein synthesis, and glucose oxidation (with and without added insulin).

Hemorrhagic shock-induced resistance and alterations in muscle leucine oxidation and protein synthesis occurred in the pancreatectomized and adrenalectomized groups, despite prevention of shock-induced alterations in pancreatic or adrenal hormone secretion through the constant infusion of insulin (Table I). The suppression of muscle protein synthesis by shock in the low-calorie animals was reversed by provision of adequate nutrition. Shock failed to alter further the large nitrogen losses of low-calorie animals or to induce significant nitrogen loss in well-alimented animals.

D. Effect of Nutritional Support on Hind Limb Protein and Glucose Metabolism

Further studies involving the metabolic derangements that occur after shock and trauma were carried out in our rhesus monkey hind limb model.

The early post-traumatic period is marked by protein hypercatabolism and gluconeogenesis with negative nitrogen balance lasting 2 to 5 days after major uncomplicated surgery. In spite of important advances in post-operative nutrition, the potentially life-threatening protein-wasting mechanism is still poorly understood. This study evaluates the role of substrate provision on muscle metabolism in the early post-traumatic period. An animal model is used which permits the assessment of muscle uptake and release of substrates as well as nitrogen balance studies.

Four male rhesus monkeys, weighing 8.1 to 15 kilograms, were submitted to laparotomy for placement of catheters through the internal iliac vessels for sampling of external iliac arterial and venous blood; a Foley catheter was placed in the bladder through a cystostomy. Throughout the procedure and for the 24 hours following the operation (Day 1) the animals, previously acclimated to restraining chairs, received normal saline; this was followed by the

provision of total parenteral nutrition with Freamine (80 cal/kg/day) for 24 hours (Day 2). Arterial and venous blood samples were drawn from the unanesthetized animals at the end of the two periods for the measurement of glucose, FFA, lactate, pyruvate and alanine. Urine samples were collected for total nitrogen determination. The data for the two periods were compared using a paired t-test (Table III).

In the 24-hour period that followed surgery (Day 1), a net output of alanine for muscle and negative nitrogen balance were observed in all animals. The nutritional support in the second 24-hour period (Day 2) produced significant reduction or reversal of both phenomena ($p < 0.01$) and hyperglycemia ($p < 0.01$). However, the muscle handling of glucose, lactate, pyruvate and FFA was not affected (arterio-venous differences not significantly different between Day 1 and Day 2).

E. Persisting Structural Abnormalities in Liver, Kidney and Muscle Tissues Following Hemorrhagic Shock

The metabolic and structural changes occurring during hemorrhagic shock and in the immediate post-shock period have been extensively discussed in the literature, but sequential studies of the post-shock and recovery period have received much less attention. We have recently reported metabolic abnormalities of muscle and adipose tissue, persisting for a week or more after resuscitation from hemorrhagic shock. This study provides new evidence of prolonged morphologic abnormalities of hepatic, renal and muscular tissue, apparent in varying degrees from 24 hours to one week after resuscitation from shock.

New Zealand strain albino rabbits (4 to 5 kg), anesthetized with a mixture of fentanyl citrate (0.2 mg/kg) plus droperidol (10 mg/kg) (Innovar Vet) were subjected to hemorrhagic shock by withdrawal of sufficient blood (about 100 to

EFFECT OF NUTRITIONAL SUPPORT ON POST-TRAUMA MUSCLE METABOLISM

TABLE III

Animal #	GLUCOSE*		LACATE*		PYRUVATE*		ALANINE*		FFA*		N BALANCE**		
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	
1	A	6.25	22.05	13.0	15.0	.213	.153	.772	1.352	.874	2.84	-303	±296
	A-V	-8.5	-7.05	-2.56	-5.7	-.041	-.045	-.558	-.226	-.018	±.84		
2	A	6.6	16.98	1.54	0.70	.055	.075	.289	.200	.946	.910	-209	±65
	A-V	±0.4	-.21	±.21	-.80	0	-.024	-.177	-.265	-.005	-.027		
3	A	5.8	26.49	0.91	1.02	.232	.367	.142	.133	.843	.756	-167	±139
	A-V	±1.05	-.22	-.28	-1.15	-.126	±.037	-.106	±.026	-.146	±.108		
4	A	5.8	12.75	3.75	1.02	.215	.202	.383	.238	.874	.205	-635	-458
	A-V	±1.05	±1.40	±1.2	-1.15	±0.54	±.058	-.133	-.67	-.017	-.014		

*mM/L

**mg N/kg/day

150 ml) to establish an arterial blood pressure of 30 mmHg. After 30 minutes the shed blood was reinfused at a rate of 5.2 ml/min. Controls were anesthetized, but not cannulated or bled.

Three groups, each consisting of two control and five post-shock rabbits, were killed on either day 1, 3 or 8 after shock. Before killing the animals, they were anesthetized, and samples of liver, kidney and skeletal muscle (transversus abdominis) were obtained. Liver and muscle tissues were immediately fixed in a 37% formaldehyde solution, and kidneys in Bouin's solution. Muscle segments were maintained at rest length throughout excision and fixation. Three to 5- μ sections of these tissues were stained with hematoxylin-eosin and examined by light and phase microscopy, and photographed using a Zeiss micrograph photobench. A separate set of experiments was performed that was identical to those reported above except that sodium pentobarbital (Nembutal) (30 mg/kg) was used as an anesthetic. We wanted to test the possibility that the α -adrenergic blocking properties of droperidol (Inapsine) in Innovar could alter the effect of shock on tissue structure. No differences could be detected in tissue changes occurring after shock when the two anesthetic agents were compared.

The findings revealed marked structural changes, some of which became more pronounced between one and three days after shock and all of which persisted for at least three days. All of the tissue changes reverted to near normal by eight days. The specific swelling and vacuolization one day after shock; renal vacuolization and swelling of proximal tubular convolutions, culminating in widespread cellular damage three days after shock; and separation of myofibrils

and disruption of the band pattern of muscle tissue. These findings demonstrated the prolongation of the morphological abnormalities induced by shock, previously observed only in the acute phase, and the capacity for structural repair by the tissues after reversible hemorrhagic shock.

II. Diurnal Rhythms of Cortisol in the Non-Human Primate

Our most recent investigations have been in the field of biological rhythms. It is possible that perturbations in rhythmical, episodic patterns of release and degradation of hormones may be involved in the metabolic alterations observed after trauma. It is possible that stress and trauma may alter circadian endocrine rhythmicity, which may affect physiological functioning of the individual. Plasma circadian rhythms of certain hormones are known to be influenced by various external stressors. This group of studies in rhesus monkeys and baboons lay the groundwork for continued studies of the effect of trauma on these circadian endocrine rhythms.

A. The Role of Degradation in the Diurnal Pattern of Cortisol

The first protocol was designed to determine the chronobiological characteristics of cortisol metabolism. The hypothesis to be tested was that since peripheral blood concentrations are a composite of both production and metabolism, it is possible that there is also a variation in the metabolic rate of cortisol.

Rhesus monkeys were placed in restraining chairs and maintained in environmental isolation with 12 hours dark/light cycles and ad libitum food and water intake for 7-15 days. Control samples were drawn every hour for 48 hours after the adaptation period to provide an adequate number of results for a meaningful

mathematical analysis of rhythmicity. This was followed by bilateral adrenalectomy and replacement with hydrocortisone sodium succinate at a constant infusion rate of 6 ug/kg/min for the first 72 hours; the dose was then reduced to 3 ug/kg/min. On the 3rd or 5th post-operative days the sampling protocol was repeated for 48 hours or 24 hours. Cortisol was measured by radioimmunoassay. The results were subjected to non-linear regression analysis fitting sine-cosine models which could reflect both circadian and infradian cycles.

The analysis of our data from the castrated-intact primates confirmed the presence of a circadian rhythmicity of cortisol levels without modifying components. After bilateral adrenalectomy and with hydrocortisone replacement at constant rates, the temporal distribution of plasma cortisol concentrations exhibited, in the majority of the experiments, a pattern characterized by the combination of two rhythms. The first components showed a periodicity of approximately 24 hours (circadian), while a second component presented cycles with durations of less than 24 hours (infradian).

The following mathematical analysis was developed for a precise definition of these rhythms. The regression model that was used to describe the post-adrenalectomy results had the form:

$$y_t = A_0 + A_1 \cos \left(\frac{2\pi}{24} t + B_1 \right) + C^t A_2 \cos \left(\frac{2\pi}{P} t + B_2 \right)$$

where y_t = cortisol concentrations; A_0 = average of y_t ; A_1 = amplitude of the circadian cycle; A_2 = amplitude of the infradian cycle; B_1 = phase of the circadian cycle; B_2 = phase of the infradian cycle; P = duration of the infradian component; and C = damping factor applied to the infradian rhythm ($C < 1$). The first major component, i.e. $C^t A_2 \cos \left(\frac{2\pi}{24} t + B_2 \right)$, explains a damped infradian

rhythm (a cycle of less than 24 hours' duration with a fading amplitude). The total variability of the data is significantly explained by this regression model ($p < 0.001$).

The calculated averages of cortisol concentrations, the amplitudes of both rhythms, the values of the damping factors, the duration of the infradian cycles and the coefficients of determinations (R^2) are presented in Table IV. In 7 out of 8 experiments, the circadian component showed significant amplitude at $p < 0.05$, and in 6 of them the infradian rhythm showed significant amplitude at $p < 0.05$. The duration of the infradian cycles ranged from approximately 3 to 16 hours. The coefficient of determination, which indicates the proportion of variability of the data, accounted for by the regression model, ranged from 78 to 97%.

We have demonstrated that cortisol metabolism has a defined chronologic pattern composed of the combination of a circadian, and an infradian rhythm may play a role in the composite patterns of circadian cortisol rhythms observed in the intact animal. These findings must be considered when aberrations in hormone rhythms are analyzed.

B. Effect of Hemorrhage on the Circadian Rhythm of Cortisol in the Rhesus Monkey

This study was designed to determine the effect of medium volume hemorrhage (20% of blood volume) upon the circadian rhythm of peripheral cortisol levels. Five rhesus monkeys were adapted to restraining chairs in our isolation room with 12 hour light/dark cycles. Food and water were available ad libitum. After 7-10 days, catheters were implanted into both femoral veins, and the monkeys were returned to the chairs for an additional 7-10 days. Control

CIRCADIAN RHYTHM PARAMETERS OF PLASMA CORTISOL
IN THE FEMALE RHEUS MONKEY

TABLE IV

EXPERIMENT #	AVERAGE CORTISOL CONCENTRATIONS	AMPLITUDE OF CIRCADIAN CYCLE	AMPLITUDE OF INFRADIAN CYCLE	DAMPING FACTOR	DURATION OF INFRADIAN CYCLE	COEFFICIENT OF DETERMINATION
	(A ₀) (µg%)	(A ₁) (µg%)	(A ₂) (µg%)	(C)	(P) (hours)	(R ²)
1	102.53	25.26*	70.76*	0.55	7.67	96%
2	84.53	6.44*	56.15*	0.58	9.88	92%
3	215.79	111.45*	158.96*	0.93	16.08	96%
4	212.83	19.54	271.04*	0.61	11.92	92%
5	164.39	28.49*	58.67*	0.37	2.93	78%
6	105.45	29.09*	9.70	0.99	5.57	80%
7	131.80	64.26*	13.74*	0.99	7.97	97%
8	102.25	24.58*	34.45*	0.63	24.85	90%

*significant at $p < 0.05$

samples were drawn each hour for 48 hours using a withdrawal pump and fraction collector. Immediately after sampling, a volume of blood estimated to be 20% of the total blood volume, was removed and stored at 4°C in blood bags. Two days later, sampling was repeated. The blood samples were handled aseptically, and the resuspended red blood cells were reinfused during sampling. The cortisol levels were determined by the radioimmunoassay and subjected to non-linear regression analysis fitting of sine-cosine models, which would reflect a circadian cycle and any phase shifts occurring after hemorrhage.

Significant technical difficulties were encountered in attempting to establish normal baseline circadian rhythms for hormones in the rhesus monkey. Specifically, our problems included swelling of the legs when the femoral veins were catheterized, potential effects of sampling because of the small blood volume of the rhesus monkey and lack of tight control of the temporal information received in the isolation room.

Only 1 of 5 monkeys showed a circadian rhythm by statistical non-linear cosine regression analysis in the control period (Table V). The absence of circadian rhythms in the control period correlated closely with the incidence of leg-swelling which may well have represented significant stress to the animals. The 48 ml of blood removed during sampling (about 20% of the blood volume) of the animals under study also may have caused sufficient hemodynamic and metabolic response to further obscure the circadian rhythm of cortisol in these animals. Interestingly, however, a circadian rhythm was found during the post-hemorrhage period in 4 of 5 cases. The significance of this latter finding is not at all clear.

ALTERATION OF PLASMA CIRCADIAN RHYTHMS OF CORTISOL
FOLLOWING HEMORRHAGE IN THE RHESUS MONKEY

TABLE V

ANIMAL	COEFFICIENT OF DETERMINATION* R ² (%)		PHASE ANGLE (95% CONFIDENCE) INTERVAL	
	PRE	POST	PRE	POST
1	83**	66**	0.8 (.6, 1.01)	1.55 (1.25, 1.86)
2	7	58**		
3	15	75**		
4	4	6		
5	7	48**		

**Suggestive of circadian rhythm

*Coefficient of determination due to fitting the data to a cosine function.

Although, as stated, this initial work on circadian rhythms in rhesus monkeys has been fraught with a number of difficulties, we have gained much valuable technical experience in the approach to this area of investigation. We have begun to utilize this expertise in the development of a similar model in the baboon, which shows promise of being highly successful.

Two baboons have been studied, and exhibit reproducible normal rhythms over a period of a month. One preliminary hemorrhage experiment done, using the same procedure as previously described, showed no change in the circadian pattern from pre-trauma control rhythms. Thus the baboon appears to be a superior and reproducible model for the study of cortisol circadian rhythms and trauma.

III. PUBLICATIONS

1. Harlow, C.L., Hiebert, J.M., Ryan, N.T., Soeldner, J.S. and Egdahl, R.H.: Insulin sensitivity to traumatized patients. Surg. Forum 26:42-44, 1975.
- *2. Hiebert, J.M., Kieler, C., Soeldner, J.S., and Egdahl, R.H.: Species differences in insulin secretory responses during hemorrhagic shock. Surgery 79:451-455, 1976.
3. Whitten, R.H. and Egdahl, R.H.: High-dose glucose-insulin-potassium in treatment of irreversible hemorrhagic shock. Surg. Forum 27:60, 1976.
4. Clarke, J.R., Whitten, R.H., Williams, L.F., Hirsch, E.F. and Egdahl, R.H.: Elevated red-cell sodium concentration in patients in shock: sources and significance. Surg. Forum 27:47, 1976.
- *5. Ryan, N.T.: Metabolic adaptations for energy production during trauma and sepsis. Surg. Clin. N. Amer. 56:1073, 1976.
6. George, B.C., Ryan, N.T., Culver, P.J., Lowey, B.P. and Egdahl, R.H.: Contrasting effects of cycloheximide on protein turnover of human skeletal muscle versus rat diaphragm. Fed. Proc. 1977, Abstract #4806.
7. Ryan, N.T., George, B.C., Harlow, C.L., Hiebert, J.M., Egdahl, R.H.: Endocrine activation and altered muscle metabolism after hemorrhagic shock. Amer. J. Physiol. 1977; 233:E439-E444.
8. George, B.C., et al.: Persisting structural abnormalities in liver, kidney and muscle tissues following hemorrhagic shock. Arch. Surg. 113:289. Arch. Surg. 1978; 113:289-293.
- *9. Egdahl, R.H., Meguid, M.D. and Aun, F.: The importance of the endocrine and metabolic responses to shock and trauma. Crit. Care Med. 1977; 5:257-263.

10. Aun, F., Lee, A., Mattox, S., Meguid, M. and Egdahl, R.H.:
Evidence for a rhythmic variation of cortisol metabolism
in adrenalectomized primates. Surg. Forum 28:112, 1977.
- *11. Whitten, R.H., Aun, F., Meguid, M. and Egdahl, R.H.: The
role of glucagon in post-operative catabolism. Surg. Forum
25:98, 1977.
12. Whitten, R.H., Ryan, N.T., George, B.C. and Egdahl, R.H.:
The role of adrenal and pancreatic hormones in post shock
metabolism. Surgical Forum 29:40, 1978.
- *13. Aun, F., Meguid, M., Albertson, D., and Egdahl, R.H.: Cir-
cadian rhythms of cortisol: Effects of surgery. Surgical
Forum 29:113, 1978.

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